Garnett, Inc

Watkinsville, Georgia USA

SeaKleen®

The Natural Solution for Ballast Water Treatment ™

What is SeaKleen® and How Does it Work?

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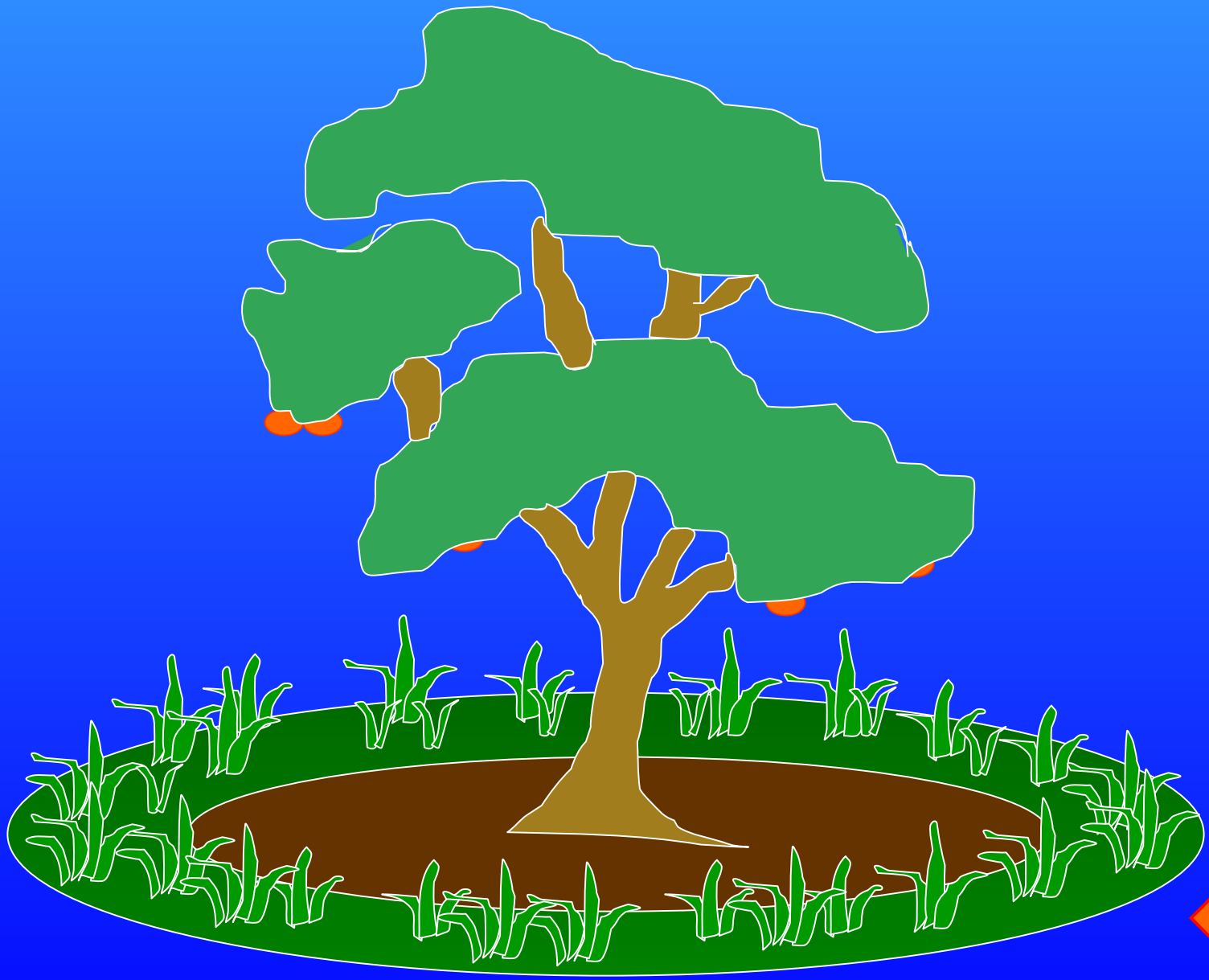
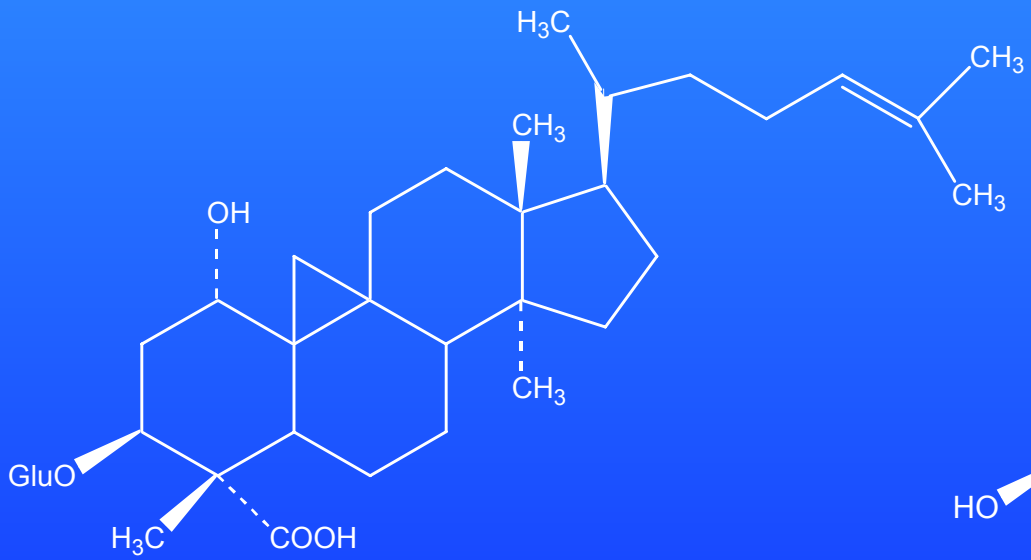
New Fairfield, Connecticut

3 University of Maryland
Chesapeake Biological Labs
Solomons, Maryland

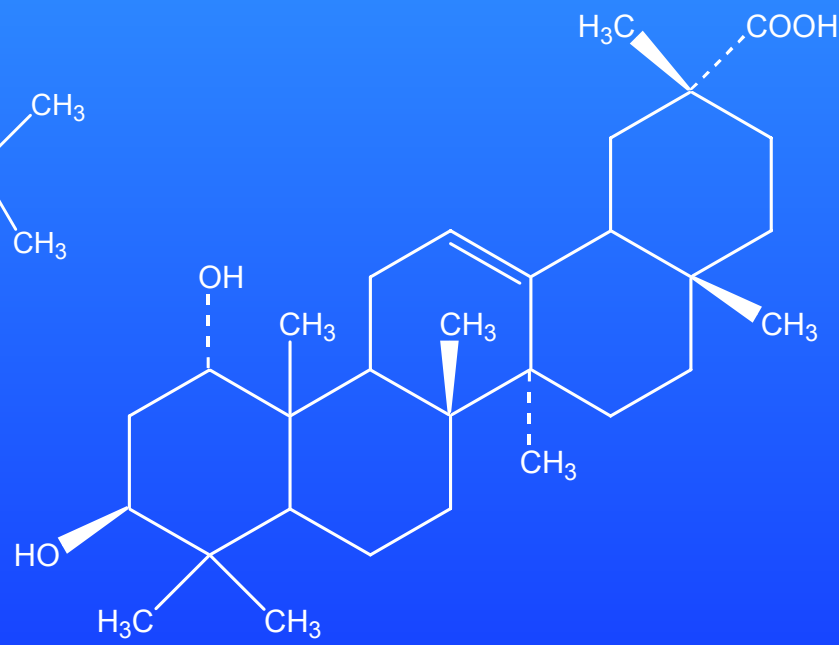
4 HortResearch Ruakura
Hamilton, New Zealand

Combretum molle



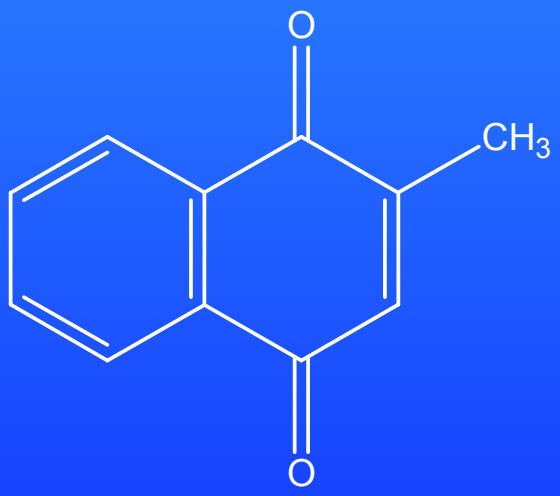


Mollic Acid



Imberic Acid

Vitamin K



Vitamin K_3
Menadione

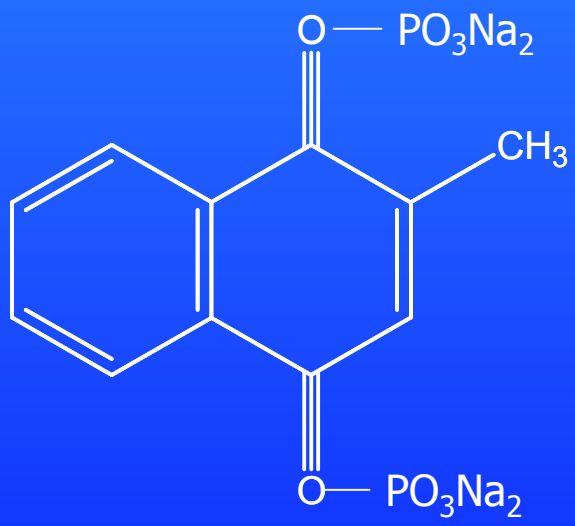
Vitamin K_1 and K_2
R = isoprenoid or isopentyl units

Black Walnut

Menadione (Vitamin K_3) Isolated from *Juglans nigra* and *Juglans regia*

Binder, RG, Benson, ME, Flath, RA
Phytochemistry *28*(10) 2799-2801, **1989**

Pharmaceutical Derivatives of Vitamin K_3



Diphosphate Derivative of Vitamin K3
Synkayvite ®
Roche (USA)

Fresh and Saltwater Organisms Controlled by Seakleen®

Phytoplankton Species : *Isochrysis galbana* (T. Iso), *Neochloris* sp., *Chlorella* sp.

Toxic Dinoflagellates: *Gymnodinium brevi*, *Prorocentrum minimum*

Dinoflagellate cysts: *Glenodinium foliaceum*

Zebra mussel larvae (*Dreissena polymorpha*)

Sheepshead minnow eggs and larvae (*Cyprinodon variegatus*)

Fathead minnow larvae (*Pimephales promelas*)

Mysid shrimp larvae (*Neomysis americana*)

Grass shrimp larvae (*Palaemonetes pugio*)

Copepods (*Eurytemora affinis*, *Tisbe* sp.)

Spiny water flea (*Bythotrephes cedarstromi*)

Benthic amphipod (*Leptocheirus plumulosus*)

Protozoans

Bacteria (*Escherichia coli*, *Vibrio fisheri* {surrogate for cholera})

Considerations for Biocides

- Effective
- Economical
- User Friendly
- Environmentally Safe

Mussel Larvae Test of Seakleen®

Mussel (*Mytilus galloprovincialis*) larvae test using Static 48-Hour Exposure to Seakleen® 80:20 and 0:100 under differing light exposure regimes [1,2]

Test Solutions aged for 48 hours in either Total Darkness or under Light Conditions

[1] x:y are x = Menadione Sodium Bisulfite and y = Menadione Wettable Powder

[2] Based on average initial count of 247 embryos per 10 ml sample

Mussel Larvae (*Mytilus galloprovincialis*) Test of Seakleen® 80:20

Concentration ppm	Aged 48 hrs Darkness Bioassay Dark % Mortality	Aged 48 hrs Darkness Bioassay Light % Mortality	Aged 48 hrs Light Bioassay Light % Mortalilty
0.5	100**	100**	100**
0.2	0	7.3	4.3
0.1	0	1.6	0
0.05	0	0	0
Control	0	0	0

Sharp Decline in Effect

** $p \leq 0.05$

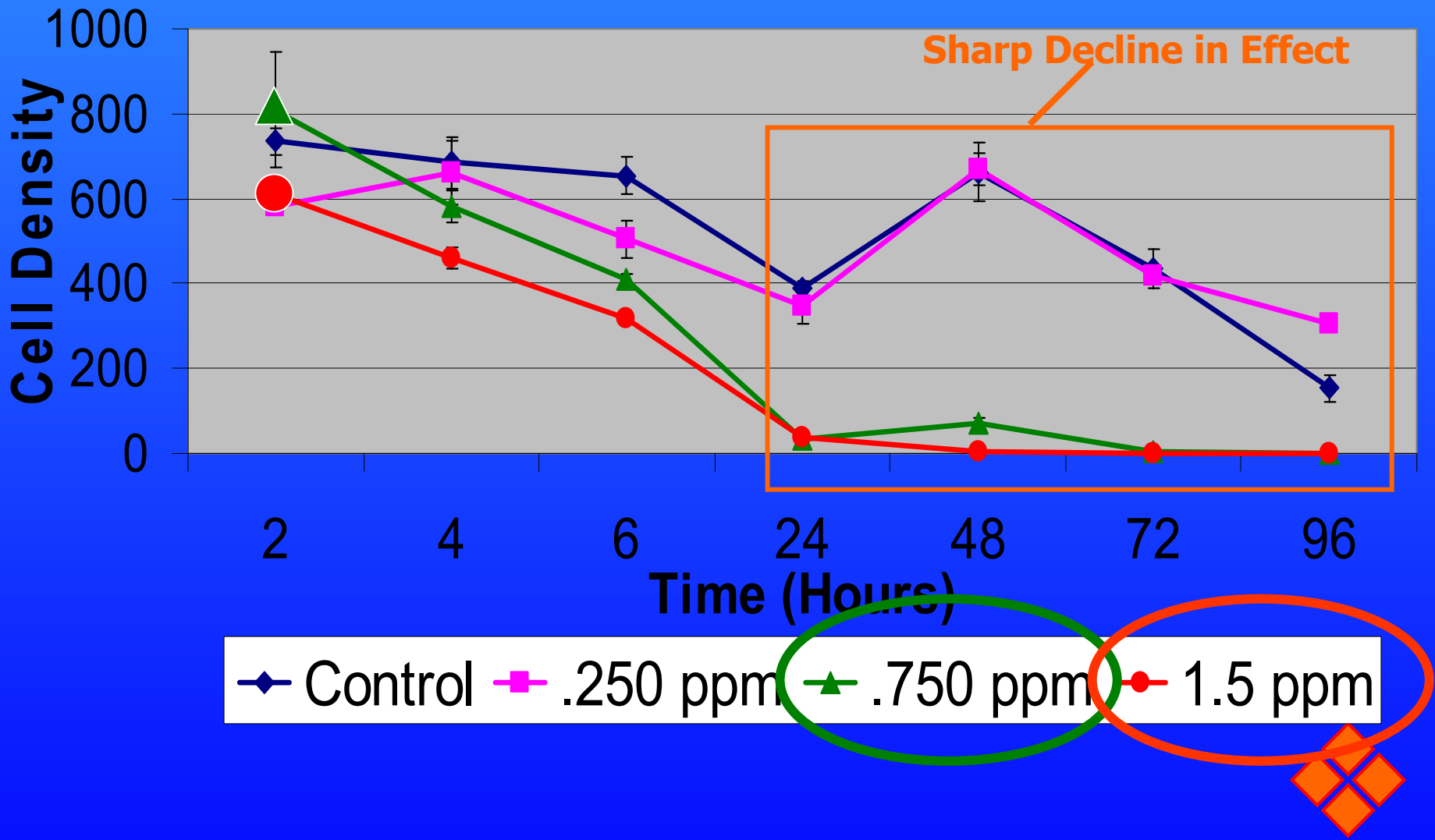
Mussel Larvae (*Mytilus galloprovincialis*) Test of Seakleen® 0:100

Concentration ppm	Aged 48 hrs Darkness Bioassay Dark % Mortality	Aged 48 hrs Darkness Bioassay Light % Mortality	Aged 48 hrs Light Bioassay Light % Mortalilty
0.5	100**	94.4**	69.9**
0.2	25.3**	3.7	8.0**
0.1	1.7	0	0
0.05	0	0	0
Control	0	0	0

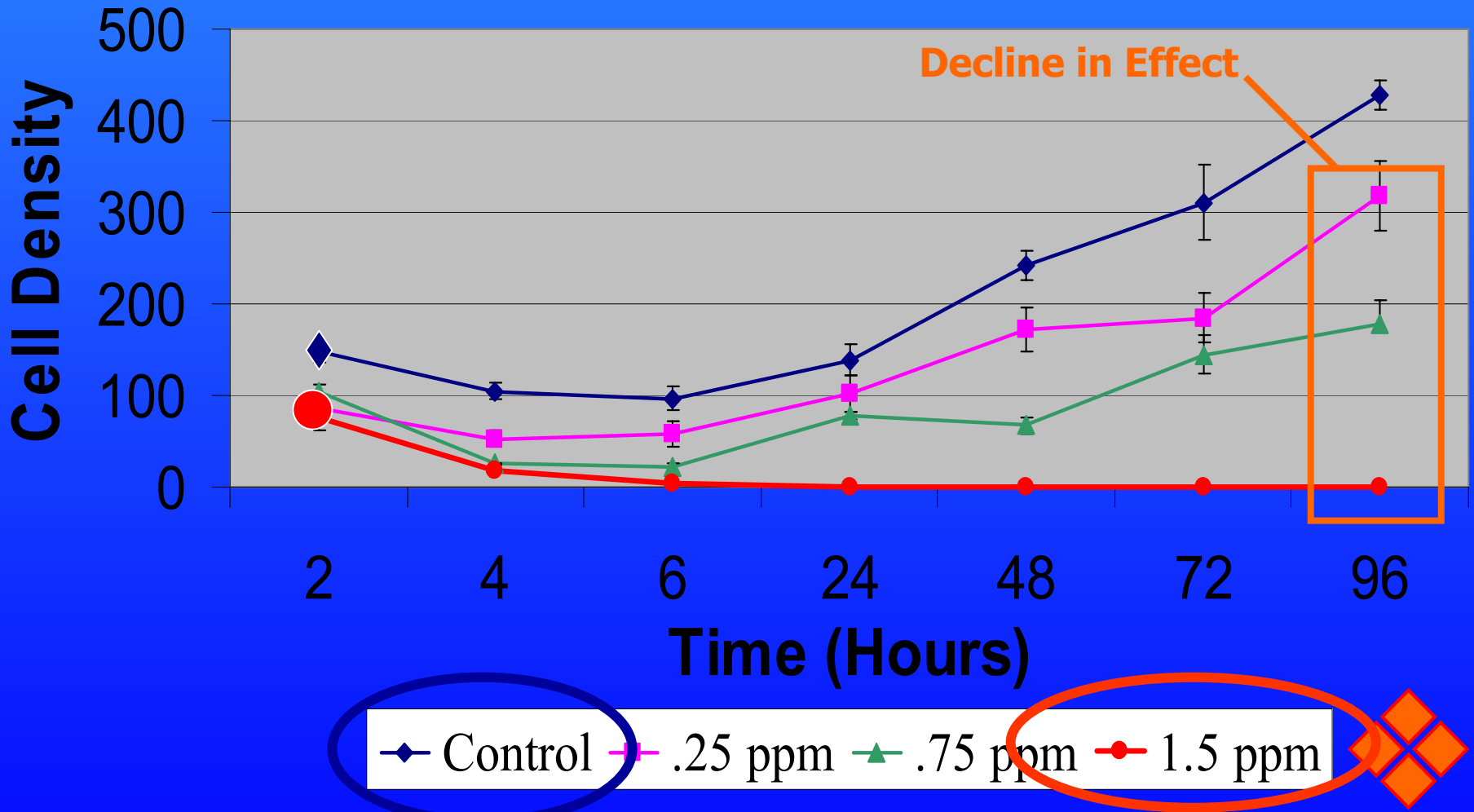
Sharp Decline in Effect

** $p \leq 0.05$

Isochrysis galbana
Exposure to Seakleen®



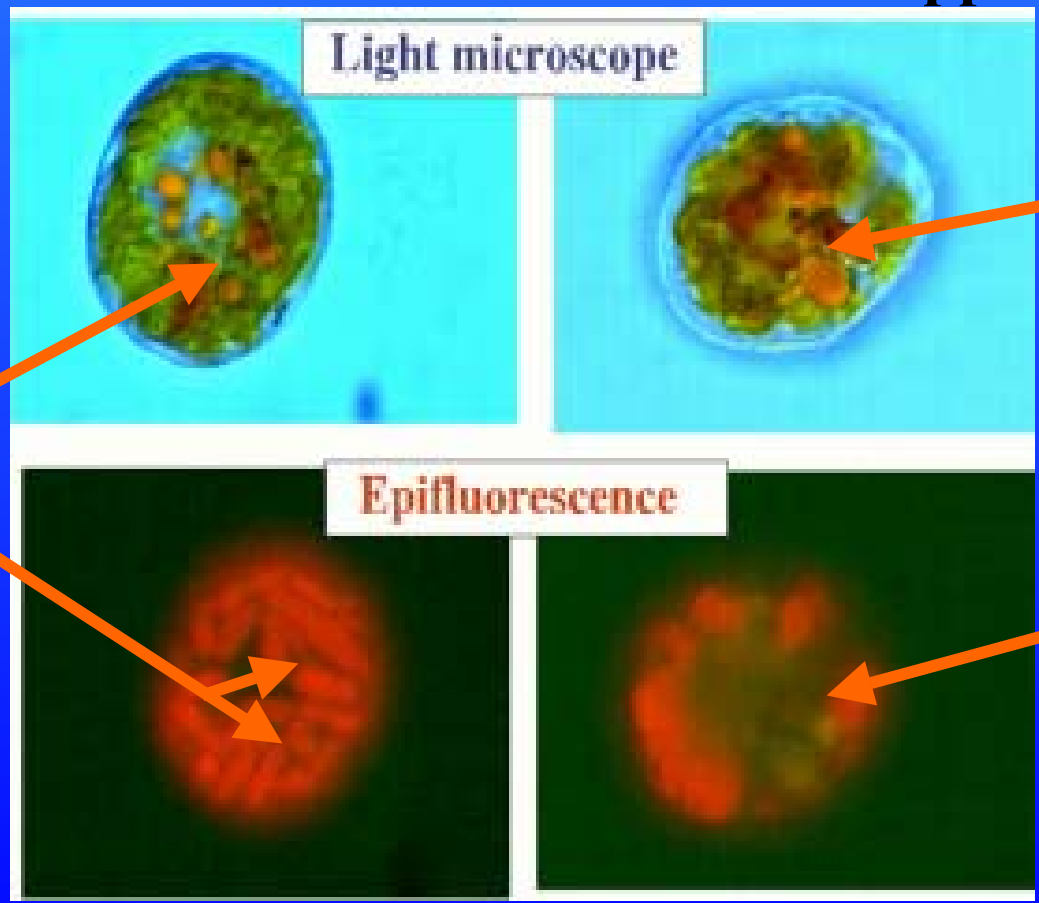
Glenodinium foliaceum
Exposure to Seakleen®



Glenodinium foliaceum cysts
2 Hours after Exposure to Seakleen®

Control (No SK®)

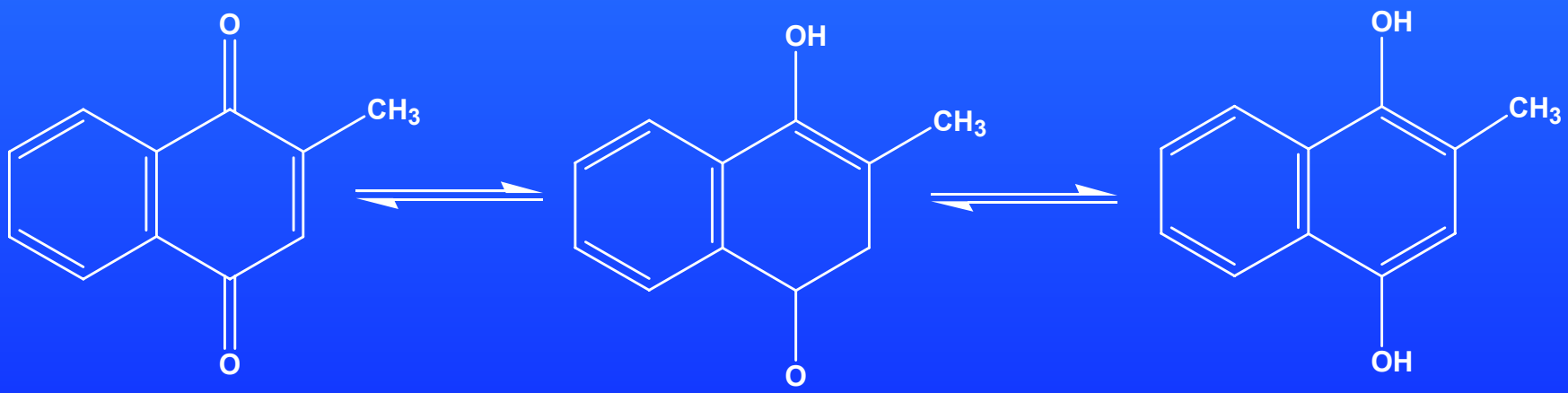
Treated with Seakleen® 2 ppm



Dilution Effect on Toxicity

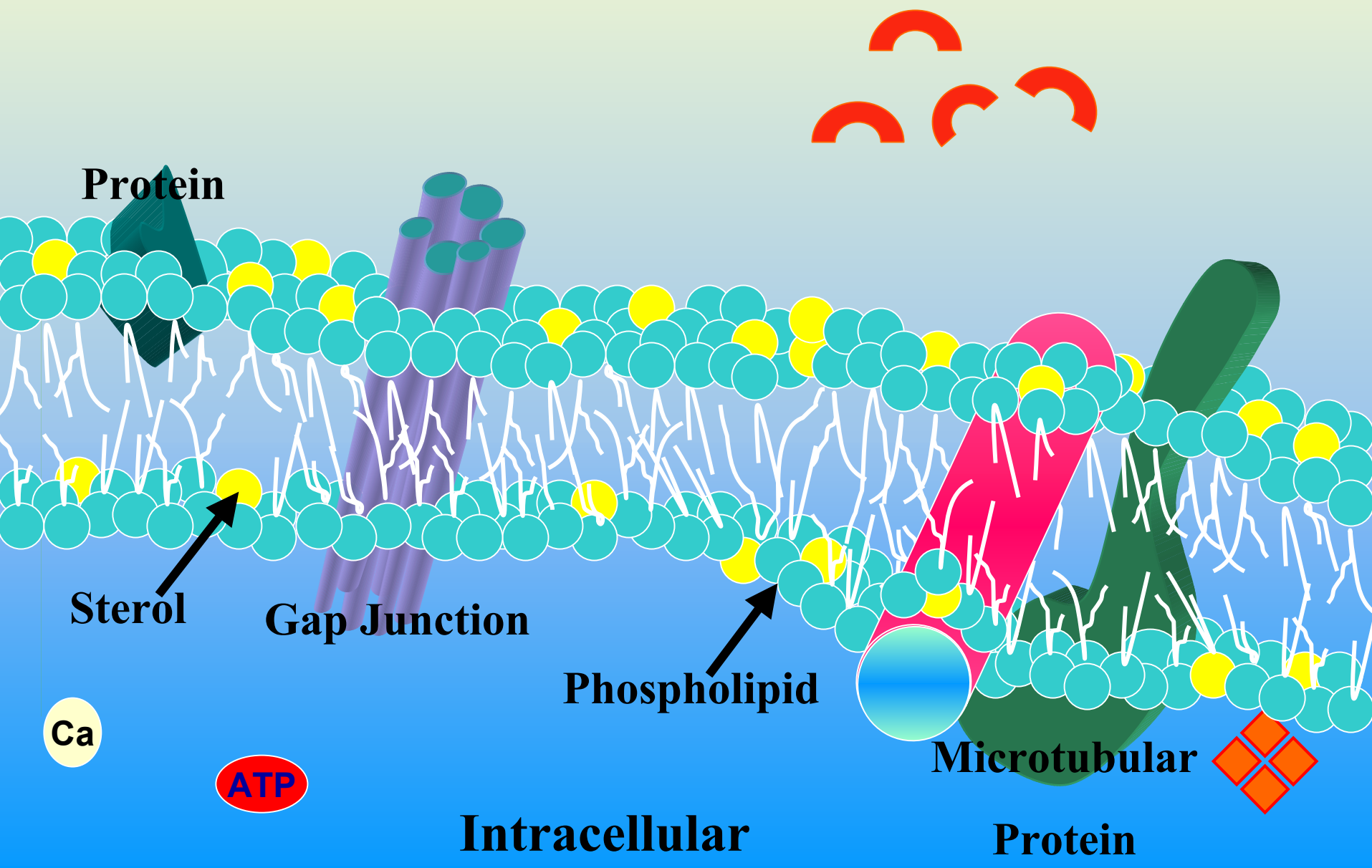
- In most cases, a 1 or 2 fold dilution causes loss in toxicity.
- Release from a ship has a multiple dilution effect.
- This is proportional to the distance from the site of release.

Potential Mechanism of Biological Action



$H^+ + e$

Quinones can oxidize cell proteins



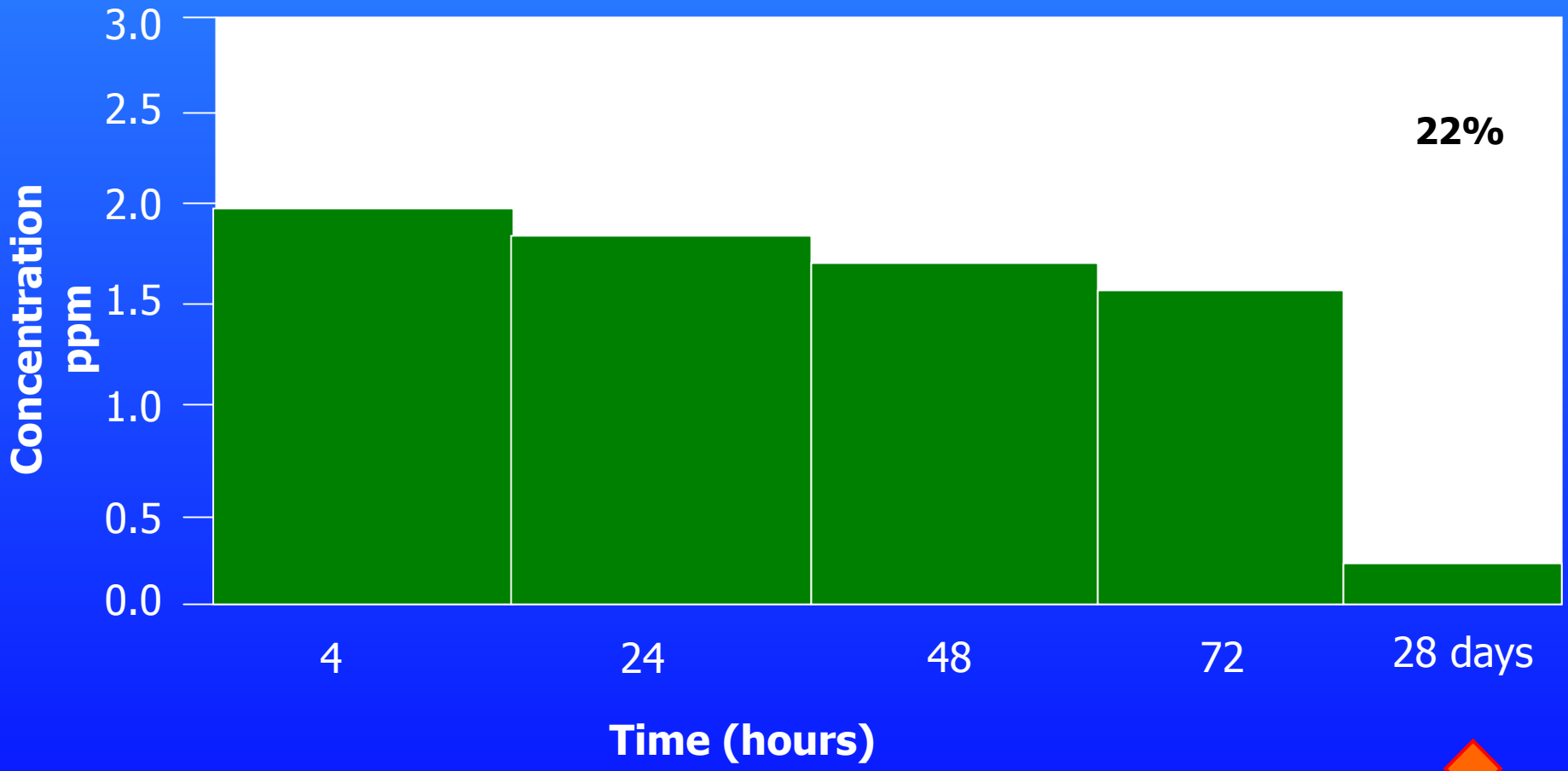
Fate of Vitamin K_3

- Dark Conditions (ballast tank)
- Light Conditions (release from ballast tank)
- Exposure to Living Aquatic Organisms

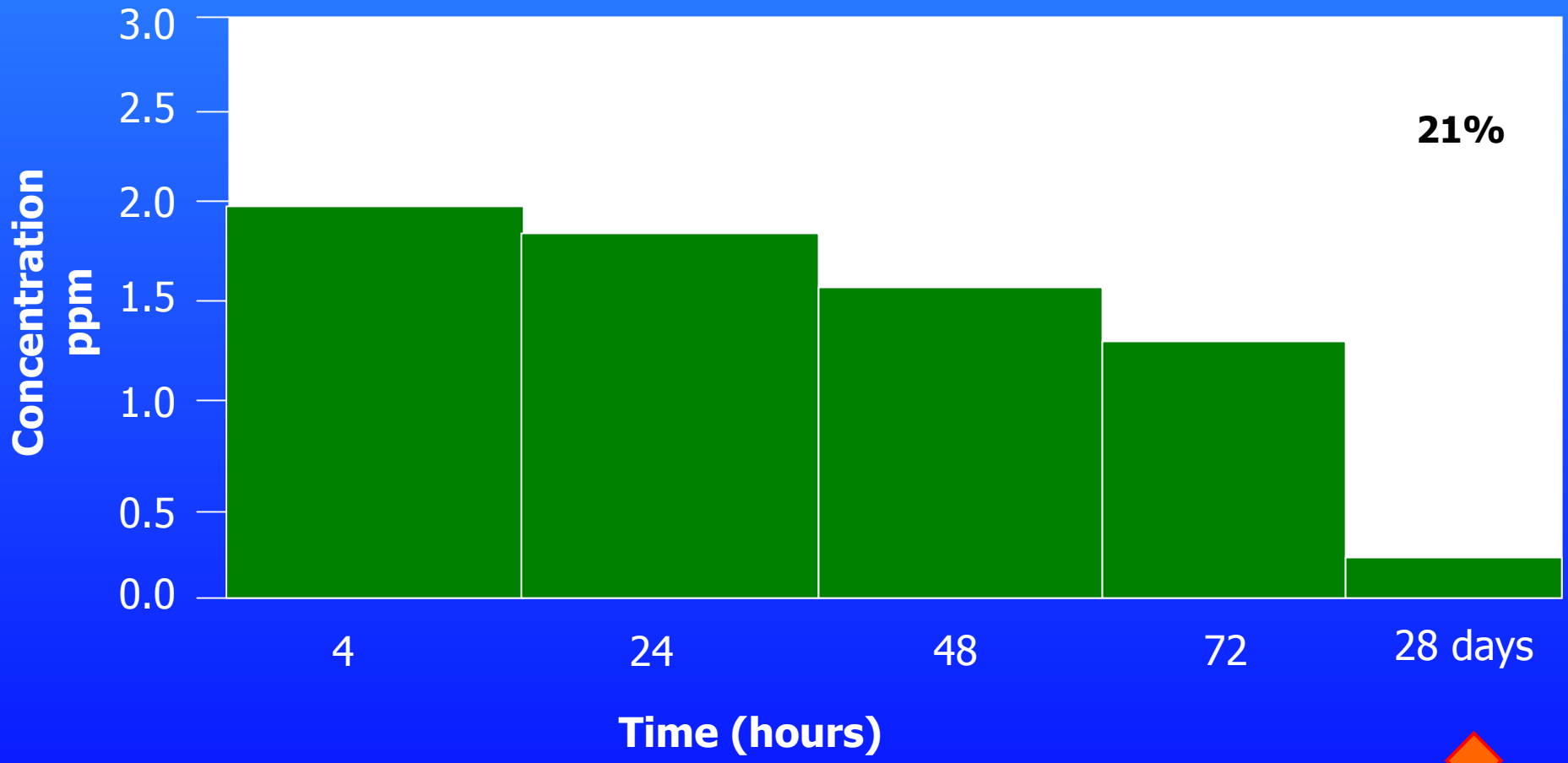
HPLC (high pressure liquid chromatography) was used to Monitor the Degradation of Menadione.

0.56 ppb Detection Limit

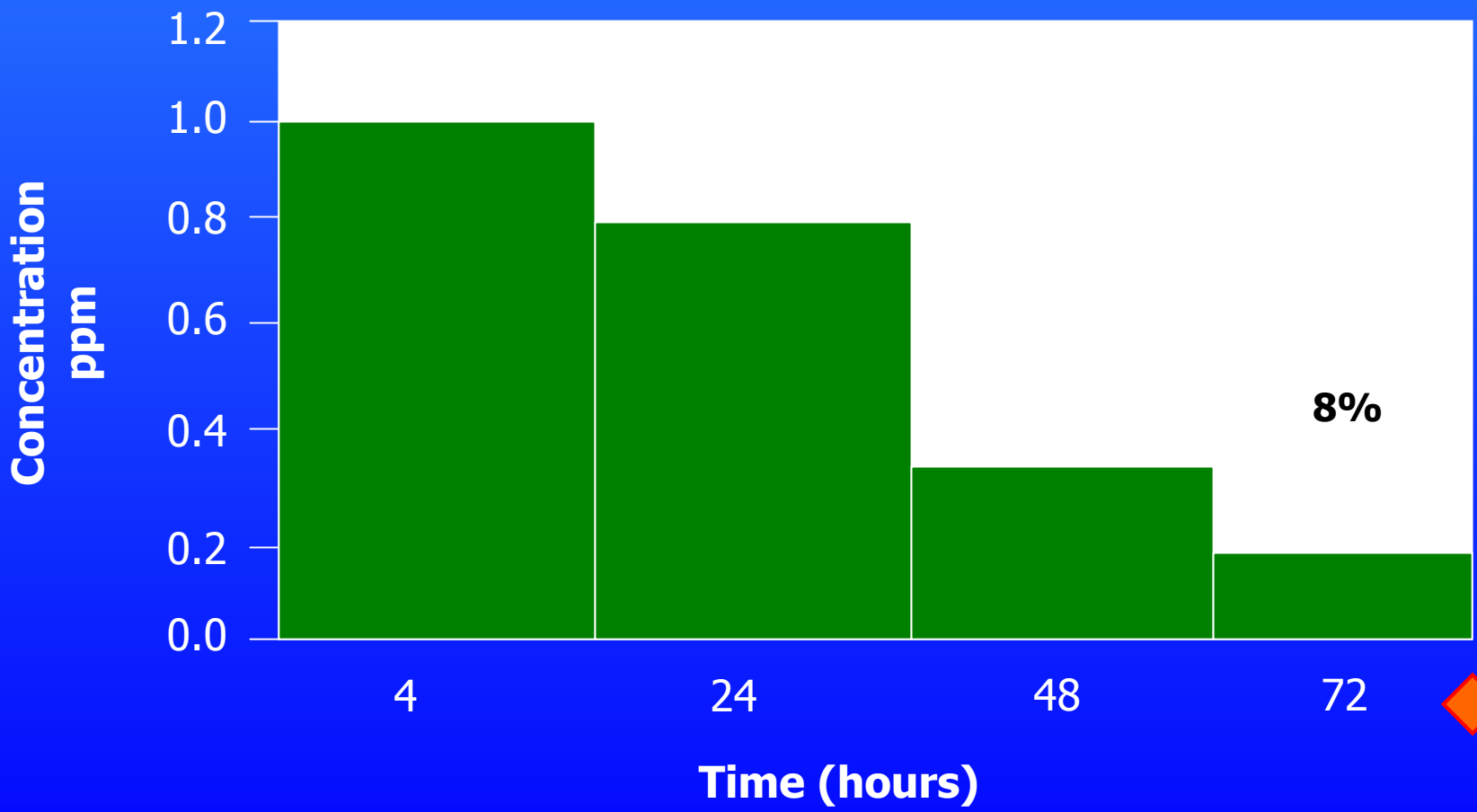
HPLC Analysis of SeaKleen®
in River Water Exposed to Darkness



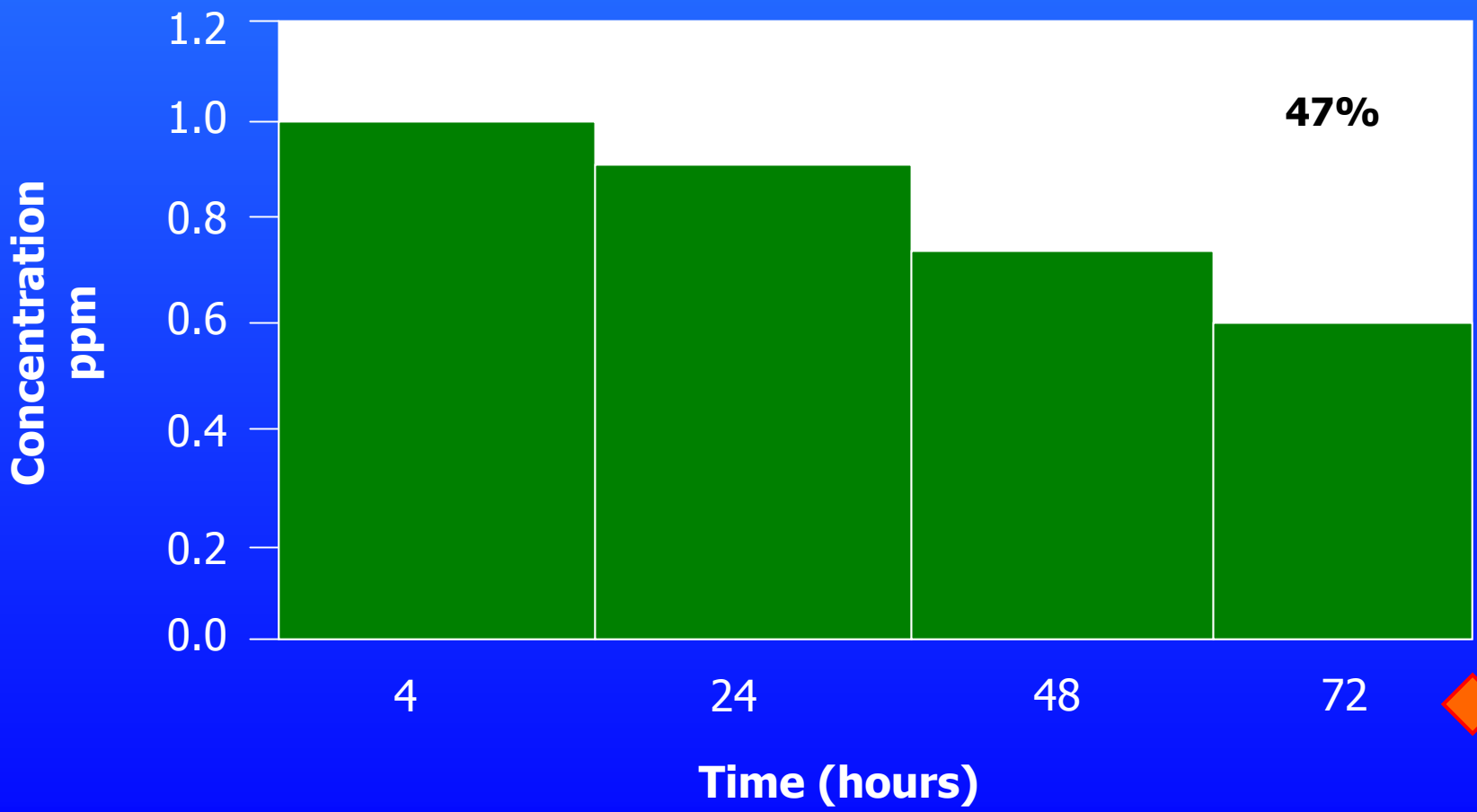
HPLC Analysis of SeaKleen®
in Sea Water Exposed to Darkness



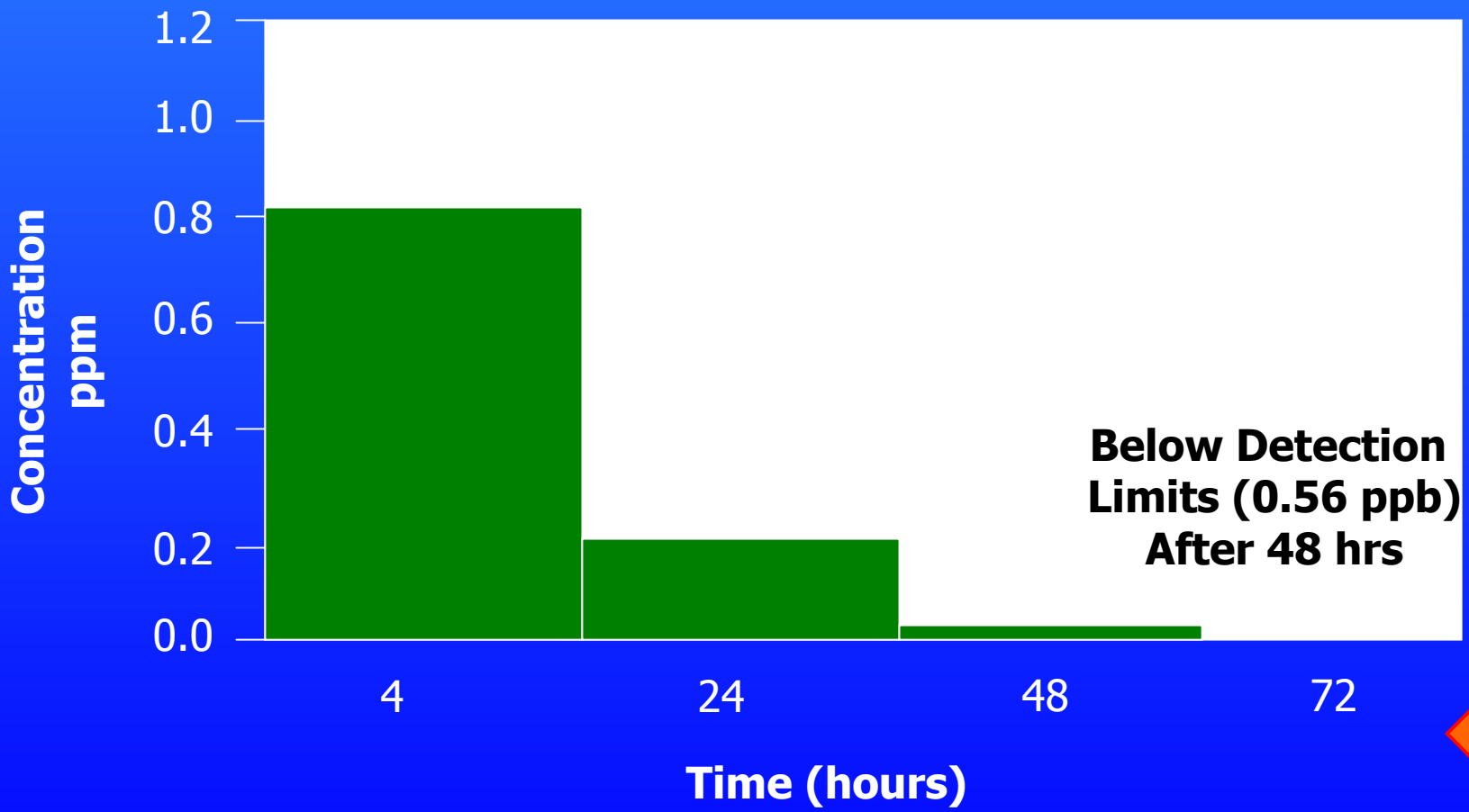
HPLC Analysis of SeaKleen®
in River Water Exposed to Sunlight



HPLC Analysis of SeaKleen®
in Sea Water Exposed to Sunlight



HPLC Analysis of SeaKleen® in River Water Exposed to Aquatic Organisms (i.e., Blue-Green Algae)



Present Agricultural Use of Menadione

Nutritional Supplement for Vitamin K Deficiency

- Chicken and Turkey Feed
- Swine (growing and finished)
- Cattle (dicoumarol)
- Duck, Pheasant, Geese
- Fish (catfish)

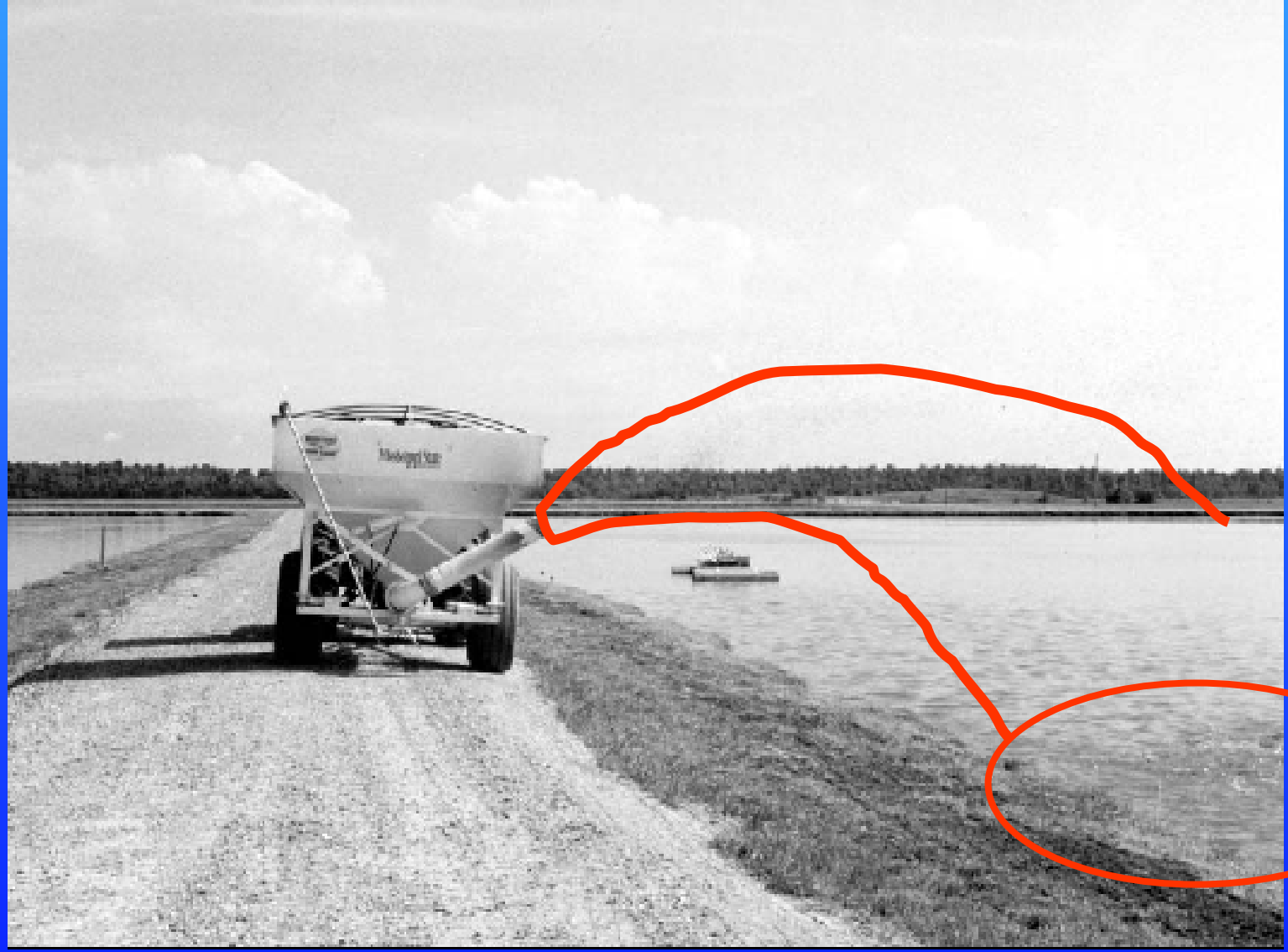
21 CFR (Code of Federal Regulations) Chapter 1 (4-01-02)

FDA Final Ruling; Federal Register, Volume 64 Number 166, 8/27/99

National Research Council

Examples of Various Feeds (includes Menadione 4.4 ppm)

Robinson, E.H., Menghe, H.L., Manning, B.B. "A Practical Guide to Nutrition, Feeds, and Feeding of Catfish." Mississippi Agricultural Forestry and Experiment Station. October 2001.



Catfish Being Fed from a Feeder Dispensing Food with Menadione (4.4 ppm)

Robinson, E.H., Menghe, H.L., Manning, B.B. "A Practical Guide to Nutrition, Feeds, and Feeding of Catfish." Mississippi Agricultural Forestry and Experiment Station. October 2001.

Catfish Feeding on
Food with Menadione (4.4 ppm)

Robinson, E.H., Menghe, H.L., Manning, B.B. "A Practical Guide to Nutrition, Feeds, and Feeding of Catfish." Mississippi Agricultural Forestry and Experiment Station. October 2001.


K - V I T
Crema
Clarificante
Clarifying
Cream
Crème
Eclaircissante
Creme
Clarificante
K
1% VITAMINA K
VITAMIN K

Acknowledgements

Vitamar, LLC
Memphis, Tennessee

Dr. Kevin Schrader
USDA
National Center for Natural Products
University, Mississippi

Hyde Marine, Inc
Cleveland, Ohio

SeaKleen®

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